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VALIDATION OF GAS CHROMATOGRAPHIC SYSTEM FOR ESTIMATION OF ORGANIC VOLATILE IMPURITIES IN HYDRO-ALCOHOLIC FORMULATION

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ABSTRACT

Separation methods occupy an important place in the array of available analytical techniques, depending on the nature of the compounds. Gas chromatography methods continue to be used to a large extent, especially in automated routine controls. The use of specialized injection and detection methods has further increased its field of applications.

The synthesis of an active pharmaceutical ingredient (API) normally consists of several synthetic steps. Process-related impurities can be formed at any step and could ultimately appear in the final drug substance, particularly in the scale-up drug candidates. Impurities must be controlled because of their potential toxicity. Impurity control is a continuing concern of regulatory agencies and the pharmaceutical industry. The International Conference on Harmonization (ICH) was formed in the 1990s to coordinate the technical requirement for the registration of pharmaceuticals in the European Union, Japan and the United States. ICH has issued the guideline "Impurities in New Drug Substances, " recommending that, for a maximum daily dose of less than or equal to 2 g per day, any impurity at the 0.10% level (or 1 mg per day intake, whichever is lower) must be identified. The Food and Drug Administration (FDA) has adopted the ICH guidelines and has published the guidelines in the Federal Register.

Key words : Validation, Hydro-alcoholic Formulation, Organic volatile impurities

INTRODUCTION

Impurities in pharmaceuticals are unwanted chemicals that are remaining with the active pharmaceutical ingredients (APIs) or developed during formulation, or upon aging of both API and formulated APIs to medicine. The presence of these unwanted chemicals

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even in small amount may influence the efficacy and safety of the pharmaceutical product. The control of pharmaceutical impurity is a critical issue. Residual solvents in pharmaceuticals (commonly know as organic volatile impurities or OVIs) are organic volatile chemicals that are either used or produced during the manufacturing of active pharmaceutical ingredients, excipients and drug products and may be hazardous to human health. Residual solvents have no therapeutic benefits but may be hazardous to human health and to the environment, they are either not present in the products or are present only below acceptable levels. However, their acceptances limit and classification vary among the three major pharmacopoeias, USP, PhEur and JP. The term "tolerable daily intake" (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and the term "acceptable daily intake" (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The new term "permitted daily exposure" (PDE) is defined in the guideline as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADI's of the same substance. They were evaluated for their possible risk to human health and placed into one of three classes as follows :

Class I : Solvents to be avoided

Class II : Solvents to be limited and

Class III : Solvents with low toxic potential.

Residual solvents are solvents that are used during the manufacturing process and may be detected after the product is in its final form. Some of the common solvents are benzene, ethanol, toluene, chloroform, 1, 4-dioxane, methylene chloride and trichloroethylene. Residual solvent in the active ingredient or drug product can come from many different stages in the manufacturing process (active substance granulation, milling, or drug product coating). Because the toxicity of most solvents has been well investigated, it is fairly easy to select appropriate control for residual solvents that may be found in the final dosage form. The most common technique for measuring residual solvents is gas chromatography (GC) because of the small size and volatile nature of solvent molecule. The amount of residual solvent can then be calculated using the peak responses.

EXPERIMENTAL

Chromatographic system- consisted of –

ESHIKA MICROPROCESSOR, Gas Chromatography.

Chemicals and reagents

1. Water (HPLC grade)
2. Methanol (HPLC grade)
3. Ethanol (AR grade)
4. Isopropyl alcohol ((HPLC grade)
5. Methylene chloride (GR. grade)
6. Chloroform (AR grade)
7. 1, 4-Dioxane (AR grade).

All the solvents and chemicals used in works were either AR or HPLC grade. Whatman filter paper No. 41 was used through out the experiment.

Chromatographic condition

Following gas chromatographic specifications were selected

(a) Column oven

- Inner volume : 28 W × 280 H × 184 D mm, 14.0 Liters
- Column pressure : 30 kpa
- Temp. range : 70 º C (isothermal)
- Temp accuracy : + 1% of the temp. Kelvin unit
- Overheat protection : Up to 300ºC

(b) Mobile phase

- Mobile phase : Nitrogen
- Flow rate : 10 mL/min
- Linear velocity : 20 cm/s

(c) Injection port

- Temp. range : 150º C
- Injection port unit : Split injection unit as standard (split ratio 1 : 2)
- Direct injection unit

(d) Flame ionization detector (FID)

- Operational temp. range : 150 ºC
- Type : Wide range type or linear type of amplifier

(e) Column specification

- Type : Designed for volatile analysis
- Composition : Cyanpropylphenyl polysoloxane (volatile)
- Diameter : 0.5 mm I. D. x 30 m length
- Film thickness : 0.5 mm
- Polarity : Polar (BP 624)
- Operation term : 70 ºC Isothermal
- Supplier : SGE

Validation approach : Preparation of standard OVIs

All working solutions were made with HPLC grade water to get concentration of methylene chloride (500 ppm), ethanol (500 ppm), isopropyl alcohol (500 ppm). chloroform (50 ppm), 1, 4 dioxane (100 ppm) Standard Organic Volatile Impurities was prepared in combination. One micro liter of this was analyzed by gas chromatography. The analysis was repeated seven times. Results were then recorded on data sheet. The method was validated for accuracy, precision, specificity, quantification limit, detection limit and robustness. The internal standard used was methanol.

The repeatability % R. S. D. was found to be less than 2% and S. D. (±) less than 1 %. The linearity was found to be in the range (0-600)**.**

Table 1 : Linearity result of ethanol

Linearity result of ethanol						
Data No.	**%**	**Conc. (ppm)**	**Ratio of analyte / I. S.**		**Mean**	**% Variation**
-	0	0	0		0	0
14	60	300	I	0.8231	0.8238	0.08
15			II	0.8246		
16	80	400	I	1.1066	1.0986	0.8
17			II	1.0907		
08	100	500	I	1.3718	1.3707	0.1
09			II	1.3697		

Cont…

Linearity result of ethanol						
Data No.	**%**	**Conc. (ppm)**	**Ratio of analyte / I. S.**		**Mean**	**% Variation**
18	120	600	I	1.6517	1.6538	0.2
19			II	1.6560		
Equation : y = 0.0027x n = 5 R^2 = 1						
Results are mean of 2 replicates. Linearity of ethanol was calculated using Internal Standard Method. The Internal Standard was methanol (500 ppm).						

Table 2 : Repeatability studies of standard organic volatile impurities

Data No.	Std. Run	Ratio of analyte peak area to that of internal standard				
		Ethanol	**Isopropyl alcohol**	**Methylene chloride**	***Chloroform***	**1.4–Dioxane**
		500 ppm	**500 ppm**	**500 ppm**	**50 ppm**	**100 ppm**
07	Replicate 1	1.3812	1.4603	0.7704	0.0483	0.2718
08	Replicate 2	1.3718	1.4531	0.7655	0.0482	0.2720
09	Replicate 3	1.3697	1.4493	0.7558	0.0483	0.2719
10	Replicate 4	1.3788	1.4553	0.7647	0.0488	0.2710
11	Replicate 5	1.3779	1.4602	0.7688	0.0484	0.2725
12	Replicate 6	1.3808	1.4615	0.7747	0.0485	0.2740
13	Replicate 7	1.3703	1.4463	0.7693	0.0486	0.2729
	Mean	1.3757	1.4551	0.7670	0.0484	0.2723
	$\pm$ S. D.	0.0050	0.0059	0.0059	0.0002	0.0009
	% R. S. D.	0.3634	0.4054	0.7692	0.4132	0.3305

Repeatability of organic volatile impurities was calculated using internal standard method. The internal standard was methanol (500 ppm).

The validated system was applied for estimation of organic volatile impurities in some popular hydro-alcoholic film coated marketed formulation :

Table 3.

Sr. No.	Name of preparation	Batch No.	Mfg. date	Sample code
1	HEPATOGARD Tab	A – 329A	DEC. 2003	H -1
		A – 924A	DEC. 2004	H -2
		A – 198A	MAY. 2004	H -3
2	EUGYNIN TAB	ANO4004	SEP. 04	E -1
		ANO4005	JUNE 04	E -2
		ANO4007	DEC. 04	E -3

Distillation of marketed preparation : (IP method III C modified)

An accurately weighed 10 g of hydro-alcoholic film coated tablets marketed formulation crushed to fine powder was mixed thoroughly. It was then transferred to the distillation flask along with 150 mL of HPLC grade water. To it, little pumice powder was added and attached to the distillation head. It was then heated up to 100ºC and about 100 mL of distillate was collected. One micro liter of this was injected and chromatograms were obtained. 500 ppm of internal standard (methanol) was added to the distillate collected. One micro liter of this was injected and chromatograms were again obtained.

It was found from the retention time of a peak in the chromatogram that ethanol and isopropyl alcohol was present as an impurity. As the permissible limit for ethanol and isopropyl alcohol is as per USP and EP, the amount of this impurity was well within the limits.

The internal standard was methanol (500 ppm).

Table 4: Gas chromatographic analysis of Hepatoguard Tab. (Solid dosage form)

Data No.	Sample	Amount aken	According to USP/EP limit of OVI in ppm					Detected OVI in ppm				
			EtOH	IPA	M. Chloride	Chloroform	1, 4 - Dioxane	EtOH	IPA	M. Chloride	Chloroform	1, 4 - Dioxane
1	H - 1	10.04 g						81.97	81.58	-	-	-
2	H – 2	10.06 g	500	500	500	50	100	82.06	81.57	-	-	-
3	H – 3	10.05 g						81.99	81.6	-	-	-
							Mean	82	81.58	-	-	-
							± S.D.	0.0472	0.0152	-	-	-
							% R.S.D.	0.0575	0.0186	-	-	-

Table 5: Gas chromatographic analysis of Eugynin Tab. (Solid dosage form)

Data No.	Sample	Amount taken	According to USP/EP limit of OVI in ppm					Detected OVI in ppm				
			EtOH	IPA	M. Chloride	Chloroform	1, 4 - Dioxane	EtOH	IPA	M. Chloride	Chloroform	1, 4 - Dioxane
4	E - 1	10.02 g						-	34.57	-	-	-
5	E - 2	10.05 g	500	500	500	50	100	-	35.05	-	-	-
6	E - 3	10.06 g						-	34.91	-	-	-
								Mean	34.84	-	-	-
								± S.D.	0.2468	-	-	-
								% R.S.D.	0.7083	-	-	-

RUSULTS AND DISCUSSION

The system was validated for each of the solvent as per the guidelines of the ICH.

The values are presented below.

Table 6

Compound	*Validation Parameter*				
	Linearity range (ppm)	**r^2**	**Repeatability % RSD (n=7)**	**LOD and LOQ (ppm)**	**Acceptable limits (ppm)**
Ethanol	0 - 600	0.9999	0.3634	50	500
Isopropyl alcohol	0 - 600	1	0.4054	50	500
Methylene chloride	0 - 600	1	0.7692	50	500
Chloroform	0 - 60	0.9998	0.4132	10	50
1, 4-Dioxane	0 -120	0.9999	0.3305	5	100

Under the optimized condition, linearity range, repeatability, limit of detection and quantization and acceptable limit of the analyte were determined. The results are shown in the Table 6.

The linearity was obtained in the range 0 - 600 ppm. Correlation coefficient (r^2) varied from 1 to 0.9998.Limit of detection and quantitation in GC system for each organic volatile impurities ranges from 5 to 50 ppm and the acceptable limit according to USP/PhEur ranges from 50 - 500 ppm. The repeatability was determined by performing seven replicates from the data obtained.

% RSD was found to be 0.4054 for most of the analyte, except for methylene chloride, which was found to be 0.7692.The results obtained in system suitability experiments indicates that –

The system under the optimized condition ensures the results of acceptable quality.

The proposed validated GC system was extended for determination of organic volatile impurities in various hydro-alcoholic films coated tablet marketed formulation for different batches :

Table 7

Sr. No.	Name of preparation	Batch No.	Mfg. date	Sample code
1	HEPATOGARD Tab	A – 329A	DEC. 2003	H -1
		A – 924A	DEC. 2004	H -2
		A – 198A	MAY. 2004	H -3
2	EUGYNIN TAB	ANO4004	SEP. 04	E -1
		ANO4005	JUNE 04	E -2
		ANO4007	DEC. 04	E -3

It was generally found from the retention time of a peak in the chromatogram methanol, ethanol and isopropyl alcohols are present as organic volatile impurities.

Quantification of this peaks led to establishing concentration level

Hepatoguard Tab (H-1, H-2, H-3) : Ethanol 81.97 ppm, 82.06 ppm, 81.99 ppm and isopropyl alcohol 81.58 ppm, 81.57 ppm, 81.60 ppm

Eugynin Tab (E-1, E-2, E-3) : Isopropyl alcohol 34.57 ppm, 35.05 ppm, 34.91 ppm

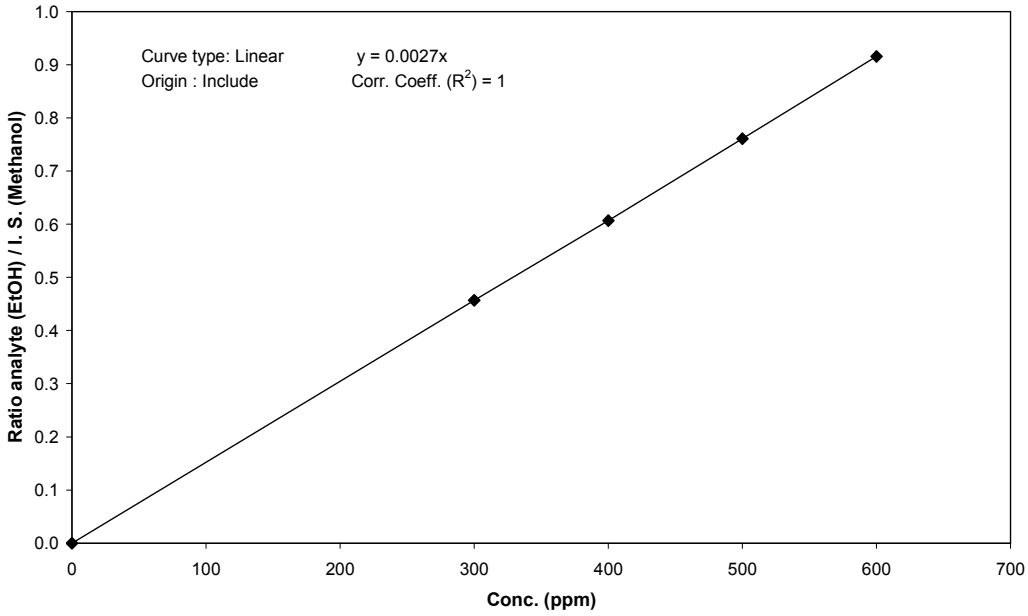
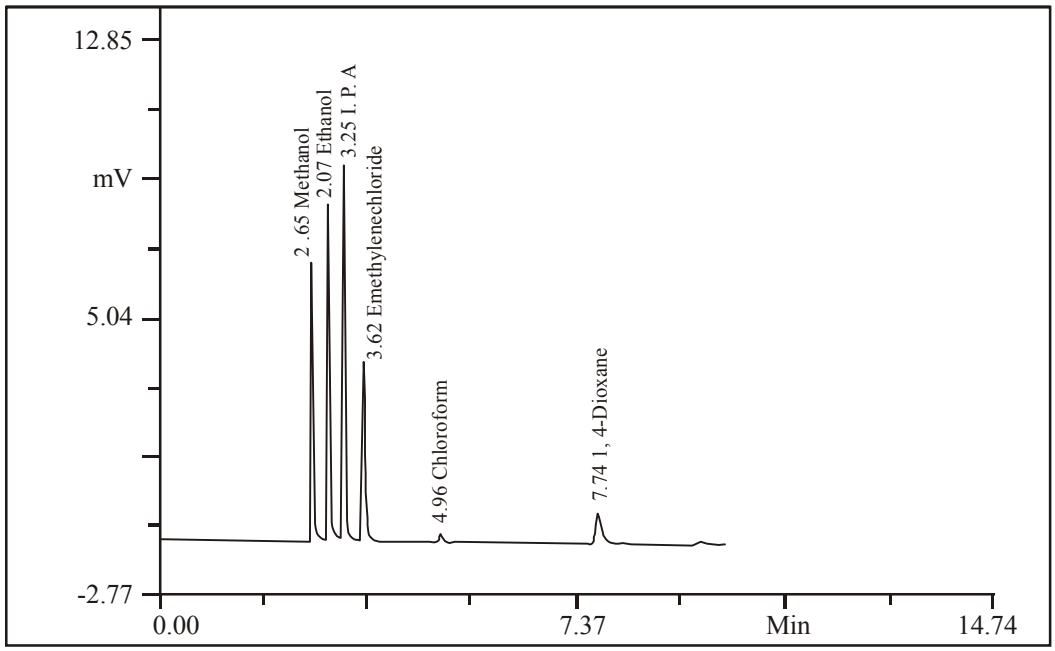


Fig. 1

Sample Name	:		Date File	:	…Ex/A1_08.Dat	
Method File	:	OVI. MET				
Detector	:	FID	System	:	GC	

Type of Analysis	:	Percent	On	Area	Height	
Pk. Width	Peak	Thrsh.	Area	Rej.	Ht. Rej	
4	30		5		4	



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.65	5267	268284	23.8380	20.3630	BV	0.043	
2	2.97	5518	368039	30.8268	27.9344	W	0.046	
3	3.25	5145	389856	28.7430	29.5903	VB	0.052	
4	3.62	2454	205397	13.7095	15.5898	BB	0.057	
5	4.96	113	12941	0.6313	0.9822	BB	0.078	
6	7.74	403	72994	2.2514	5.5403	BB	0.124	

Summary

Total Peaks 6

Mul. Factor 1, 0000

Sample amt. 100, 0000

Dilution 1, 0000

Fig. 2 : Repeatability studies of standard organic volatile impurities

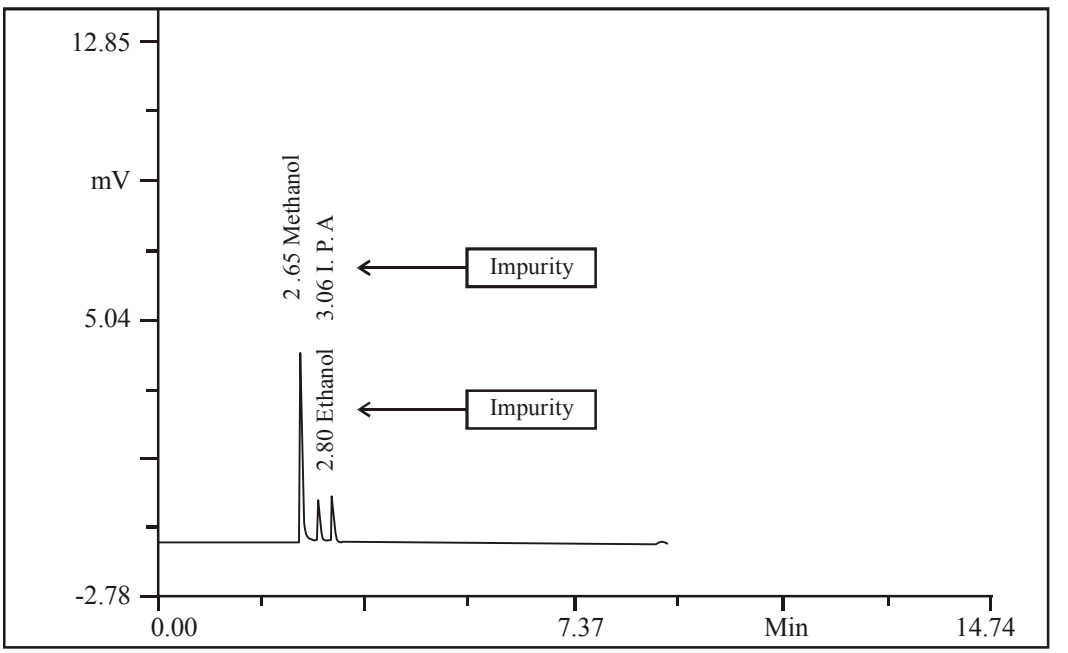
Sample Name : Date File : …Ex/A1_23.Dat

Method File : OVI. MET

Detector : FID System : GC

Type of Analysis : Percent On Area Height

Pk. Width	Peak	Thrsh.	Area Rej.	Ht. Rej
4	30		5	4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.45	3033	211412	70.8148	68.4227	BV	0.048	
2	2.78	527	47693	14.5393	15.3752	VV	0.052	
3	3.04	523	50092	14.5454	14.2011	VB	0.055	

Summary

Total Peaks 3

Mul. Factor 1, 0000

Sample amt. 100, 0000

Dilution 1, 0000

Fig. 3 : (Data No. 1)

Sample Name : Date File : …Ex/A1_24.Dat

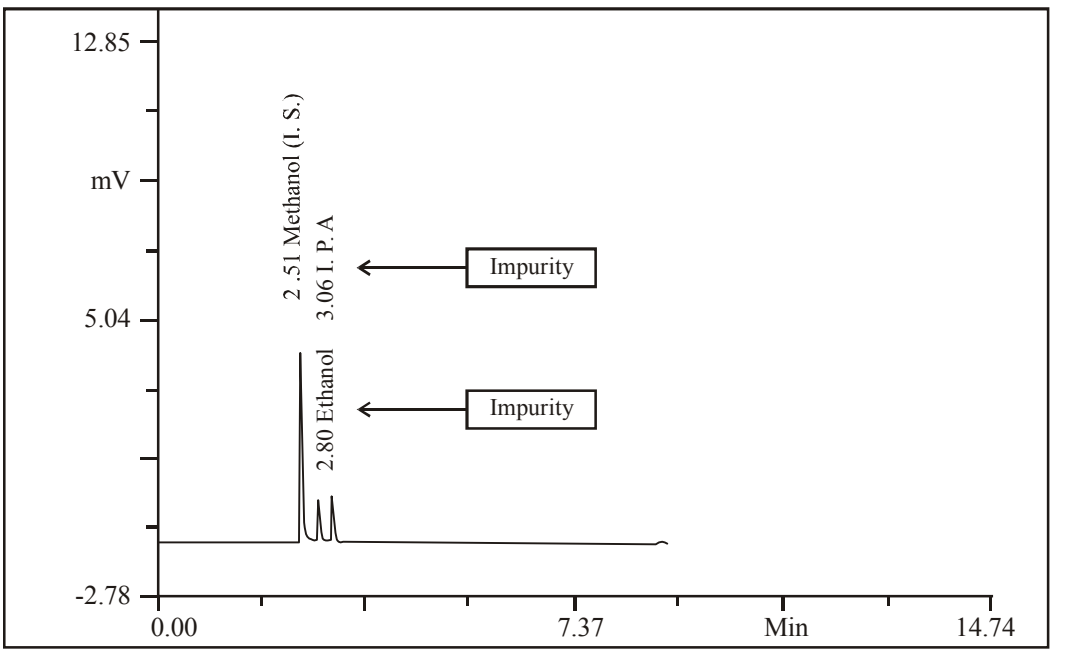
Method File : OVI. MET

Detector : FID System : GC

Type of Analysis : Percent On Area Height

Pk. Width Peak Thrsh. Area Rej. Ht. Rej

4 30 5 4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.51	3031	211416	74.1075	68.3506	BV	0.047	
2	2.80	527	47797	12.8852	15.4527	VV	0.054	
3	3.09	532	50096	13.0073	16.1967	VB	0.057	

Summary

Total Peaks 3

Mul. Factor 1, 0000

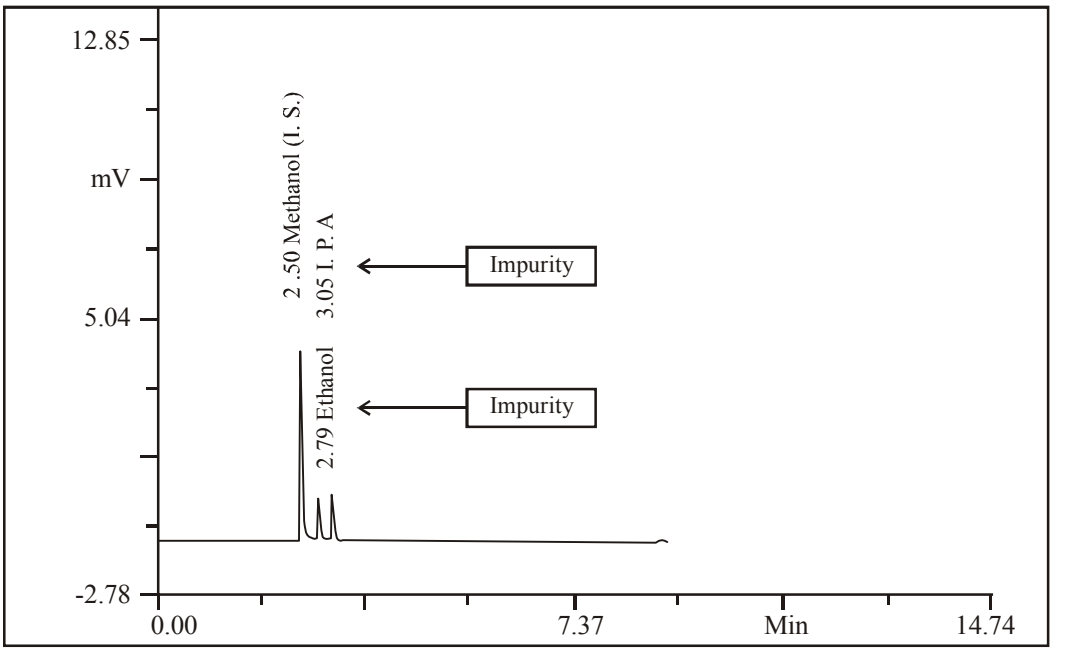
Sample amt. 100, 0000

Dilution 1, 0000

Fig. 4 : (Data No. 2)

Sample Name : Date File : …Ex/A1_25.Dat
Method File : OVI. MET
Detector : FID System : GC
Type of Analysis : Percent On Area Height

Pk. Width	Peak	Thrsh.	Area	Rej.	Ht. Rej
4	30		5		4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.50	4321	211414	74.2822	68.2190	BV	0.045	
2	2.79	754	47569	12.9621	15.3721	VV	0.055	
3	3.05	742	50468	12.7557	16.3089	VB	0.057	

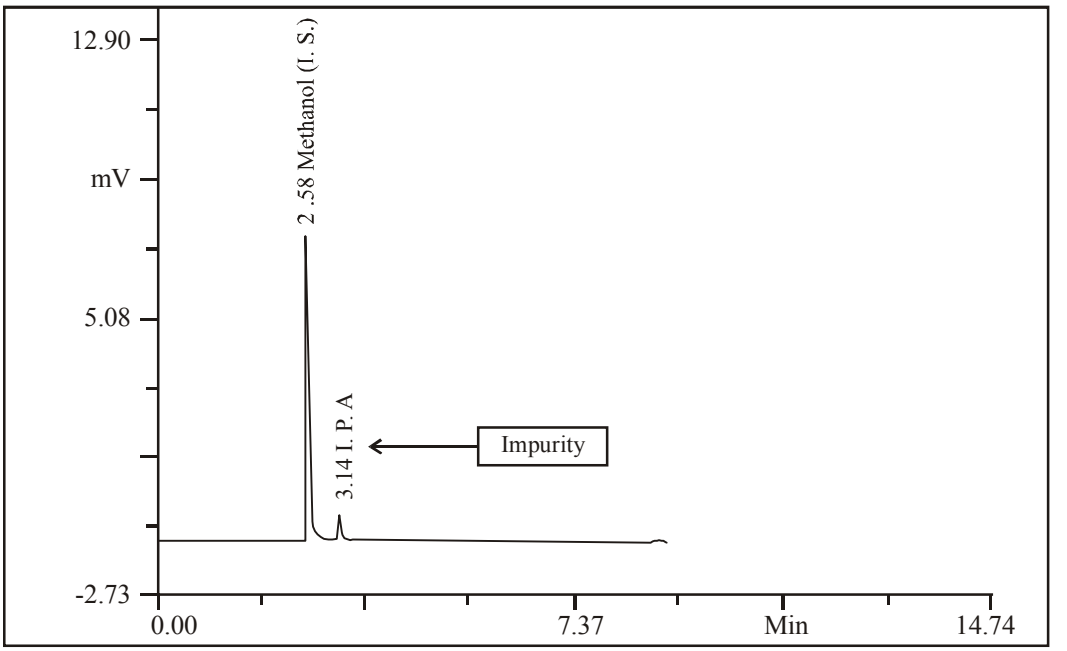
Summary

Total Peaks 3
Mul. Factor 1, 0000
Sample amt. 100, 0000
Dilution 1, 0000

Fig. 5 : (Data No. 3)

Sample Name : Date File : …Ex/A1_26.Dat
Method File : OVI. MET
Detector : FID System : GC
Type of Analysis : Percent On Area Height

Pk. Width	Peak	Thrsh.	Area	Rej.	Ht. Rej
4	30		5		4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.58	4110	257815	92.7137	90.8817	BB	0.043	
2	2.14	323	25867	7.2683	9.1183	BB	0.055	

Summary

Total Peaks	2
Mul. Factor	1, 0000
Sample amt.	100, 0000
Dilution	1, 0000

Fig. 6 : (Data No. 4)

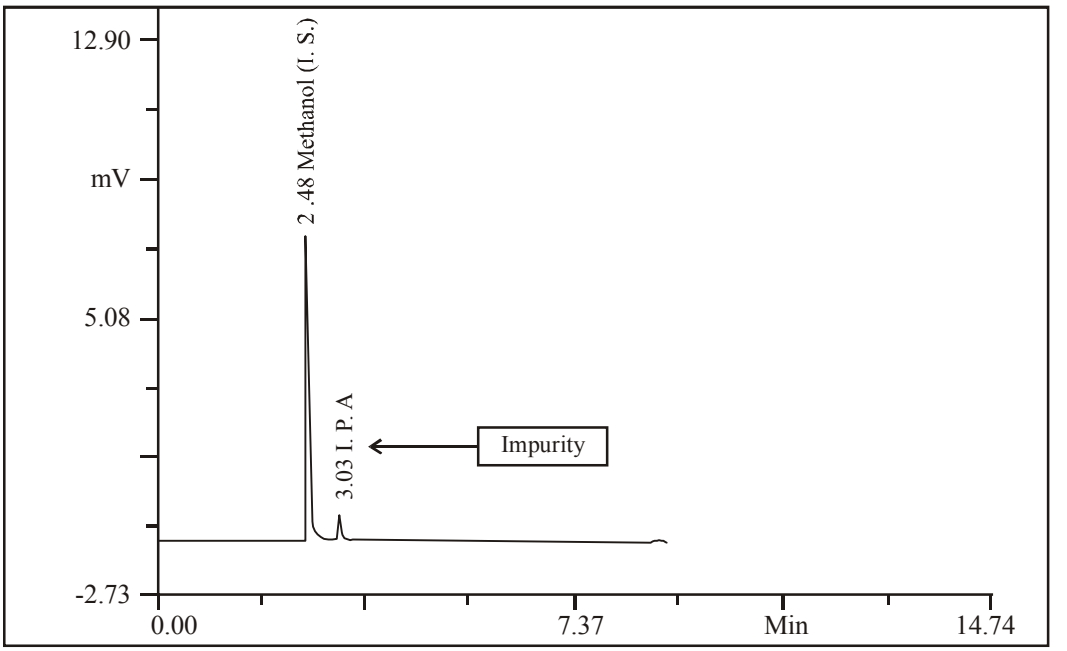
Sample Name : Date File : …Ex/A1_27.Dat

Method File : OVI. MET

Detector : FID System : GC

Type of Analysis : Percent On Area Height

Pk. Width	Peak	Thrsh.	Area	Rej.	Ht. Rej
4	30		5		4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.48	4215	257825	91.8301	90.7710	VB	0.045	
2	3.03	375	25214	8.1699	9.2290	BB	0.057	

Summary

Total Peaks 2

Mul. Factor 1, 0000

Sample amt. 100, 0000

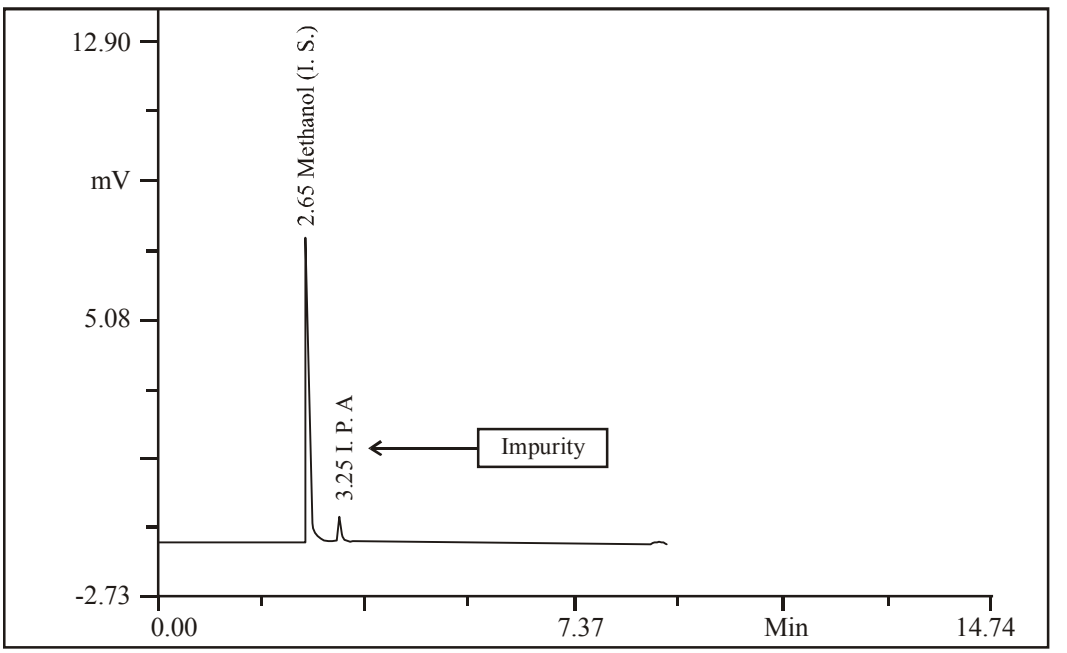
Dilution 1, 0000

Fig. 7 : (Data No. 5)

Sample Name : Date File : …Ex/A1_28.Dat
Method File : OVI. MET
Detector : FID System : GC

Type of Analysis : Percent On Area Height

Pk. Width	Peak	Thrsh.	Area Rej.	Ht. Rej
4	30		5	4



No.	R. T.	Ht.	Area	Ht. %	Area%	Pk Ty	Area/Ht	Cl
1	2.65	4525	257820	93.2990	90.8787	BB	0.042	
2	3.25	325	25877	6.7010	9.1213	BB	0.059	

Summary

Total Peaks 2
Mul. Factor 1, 0000
Sample amt. 100, 0000
Dilution 1, 0000

Fig. 8 : (Data No. 6)

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